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QTL mapping of adult plant resistance to Ug99 stem rust in the spring wheat population RB07/MN06113-8

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Abstract The emergence and spread of the Ug99 race group of the stem rust pathogen (*Puccinia graminis* Pers. f. sp. *tritici*) in the past decade have exposed the vulnerability of wheat (*Triticum aestivum* L.) to this disease. Discovery of novel and effective sources of resistance is vital for breeding resistant varieties to avert losses. The experimental breeding line MN06113-8 and cultivar RB07 developed by the University of Minnesota wheat breeding program exhibited adult plant resistance (APR) to the Ug99 race group in field tests in Kenya and Ethiopia. Both lines were found to be susceptible at the seedling stage to isolates of the race TTKSK, TTKST, and TTTSK. To dissect the genetic mechanism of resistance present in these lines, MN06113-8 was crossed to RB07 to generate 141 F_6 recombinant inbred lines (RILs). The RIL population was evaluated for APR to Ug99 in Kenya and Ethiopia over three seasons and for resistance to North American stem rust pathogen races in St. Paul, MN, in one season. The population was genotyped using high-throughput SNP genotyping assays. Composite interval mapping detected six quantitative trait loci (QTL) involved in APR to African stem rust races and three QTLs involved in stem rust resistance to North American stem rust races. One QTL located on chromosome 2B was associated with APR to stem rust races in all environments. Development of diagnostic markers linked to this gene will facilitate marker-assisted selection of resistant lines to develop varieties with enhanced levels of stem rust resistance.

Keywords Linkage mapping · Recombinant inbred lines · Stem rust · Ug99 · Genotyping by sequencing · Resistance breeding

Key message

This study reports the detection of QTL associated with resistance to the Ug99 race group of stem rust of wheat, in US spring wheat breeding germplasm.

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Introduction

One of the primary objectives of resistance breeding is the effective deployment of resistance sources that ensure durability. Constantly evolving pathogen populations challenge the effectiveness and durability of deployed resistance genes. Stem rust of wheat, caused by the fungal pathogen *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*), is one such example where the pathogen is persistently evolving to overcome host resistance. One of the oldest plant diseases known to mankind (Kislev 1982), stem rust of wheat is highly destructive and bears potential to completely destroy small-scale farm plots to millions of hectares of susceptible varieties (Roelfs 1985).

The severe threat that stem rust has historically posed to global wheat production has been magnified in recent decades by the evolution of a highly virulent race of *Pgt* typed as TTKS. The isolate of this pathogen race was first observed in Uganda in 1998 and was named Ug99 for the country of origin and the year it was evaluated (Pretorius et al. 2000). Ug99 was found to overcome lines with *Sr31*, a widely deployed stem rust resistance gene that provided effective resistance at the time. Within a few years of its discovery, this race spread toward North Africa, West Africa, and the Middle East and has potential to travel to West and/or South Asia as global wind patterns may transport the fungal spores over long distance (Hodson et al. 2011; Singh et al. 2008). Race TTKS, later named TTKSK after characterization using an expanded North American stem rust differential set (Jin et al. 2008), along with its six other related races are virulent to 85–95 % of breeding materials worldwide (Wanyera et al. 2006; Singh et al. 2011). The rapid evolution of race TTKSK and related races, the Ug99 race group, means that the pathogen is capable of defeating multiple important stem rust resistance genes, as evident by the breakdown of genes such as *Sr31*, *Sr24*, *Sr36*, and *Sr9h* (Jin et al. 2008, 2009; Singh et al. 2008; Pretorius et al. 2012; Rouse et al. 2014a). Furthermore, many of the effective stem rust resistance genes in bread wheat and its wild relatives were either ineffective against these races or unusable for practical breeding because of linkage drag associated with large translocations carrying the stem rust resistance genes linked to reduced grain quality, yield, and other desired traits (Singh et al. 2011). While this evolution of the Ug99 race group exposed the high vulnerability of wheat varieties grown worldwide, it also drew attention to the fact that the efforts in global rust monitoring and resistance breeding were not adequate to protect wheat from stem rust, making a search for durable resistance even more urgent.

Durability of rust resistance in wheat is expected to be enhanced by adult plant resistance (APR) genes (Singh 2012). APR is generally observed to condition nonspecific resistance and is characterized by low infection frequency, reduced size of urediniospores, and overall diminished urediniospore production (Stuthman et al. 2007). In the case of wheat stem rust, APR has been further described as expressed in mature plants, mostly associated with the absence of a hypersensitive response to the pathogen (Hare and McIntosh 1979), and quantitatively inherited (Knott 1982). Singh et al. (2005) discuss that pyramiding four to five APR genes can confer near immunity against diseases but may be difficult to accomplish due to large population sizes required to select transgressive segregants, and a lack of diagnostic markers associated with the resistance alleles (Singh 2012). Combining multiple seedling genes, also known as all-stage resistance genes, with or without APR genes has also been proposed and utilized to obtain durable resistance against the disease (Mago et al. 2011; Ayliffe et al. 2008; Evanega et al. 2014; Kolmer et al. 1991). Only a few stem rust APR genes have been discovered in wheat, namely *Sr2* (Knott 1968), *Sr55* (*Lr67/Yr46/Pm46*; Herrera-Foessel et al. 2014), *Sr56* (Bansal et al. 2014), *Sr57* (*Lr34/Yr18/Pm38*; Lagudah et al. 2006), and *Sr58* (*Lr46/Yr29/Pm39*; Singh et al. 2013c). Continual discovery of new genes that confer APR is vital for the protection of the wheat crop against stem rust.

The threat from the Ug99 race group has been addressed, at least partly, by wheat research teams throughout the world by screening for resistance and using the identified resistant lines in their breeding programs. Many of the genes that have recently been demonstrated to be effective to Ug99 (Jin et al. 2007) were originally identified in non-bread wheat species. Examples include *Sr32* which was identified in *Aegilops speltoides* (McIntosh et al. 1995), *Sr37* in *Triticum timopheevi* (McIntosh and Gyarfas 1971), *Sr39* in *Aegilops speltoides* (Kerber and Dyck 1990), *Sr40* in *Triticum araraticum* (Dyck 1992), *Sr44* in *Thinopyrum intermedium*, and *Sr53* in *Aegilops geniculata* (Liu et al. 2011). While wild relatives of

bread wheat are excellent sources of resistance genes, the issue of linkage drag, which occurs from introgression of genes derived from non-elite germplasm to elite breeding material, is a challenge because of the linkage of sometimes large alien chromatin blocks with the introgressed resistance genes. Breeders may hesitate to utilize such wild sources of resistance in their materials because of the time and effort it takes to select for lines with desired agronomic traits. Hence, discovery of resistant material in existing breeding programs would be a clear advantage, as crossing advanced lines with resistance to other elite lines would incorporate the resistance while also preserving desired agronomic qualities.

The wheat breeding program at the University of Minnesota develops hard red spring wheat varieties with superior agronomic performance and disease resistance. To safeguard the released varieties against a potential threat of Ug99, the program has routinely contributed dozens of advanced experimental lines each year since 2005 for resistance screening in a stem rust nursery coordinated by USDA-ARS, the International Center for the Improvement of Maize and Wheat (CIMMYT), and the Kenya Agriculture and Livestock Research Organization (KALRO) in Njoro, Kenya. The advanced experimental line MN06113-8, despite being susceptible to Ug99 races at the seedling stage, was found to exhibit APR to Ug99 races in the Njoro stem rust nursery in Kenya. The University of Minnesota wheat cultivar RB07 also displayed APR to Ug99 races in the field, but is susceptible at the seedling stage. In order to understand the genetic mechanism of stem rust resistance in these lines, they were crossed (RB07/MN06113-8) to generate a biparental recombinant inbred line (RIL) population segregating for APR to Ug99 races.

In this study, we map the Ug99 race group resistance segregating in the RB07/MN06113-8 RIL population. We also estimate the genetic effects of the detected loci and trace their origin. We hypothesize that some of the QTL regions detected in this study have not been previously identified.

Materials and methods

Plant materials

A mapping population of 141 recombinant inbred lines (RILs) was developed via advancing the F_2 genotypes using the single seed descent method by crossing 'MN06113-8' and 'RB07,' both hard red wheat lines with spring growth habit. The line RB07 has the pedigree Norlander (PI 591623)/HJ98 (Busch et al. 2000) and was developed by the University of Minnesota Agricultural Experiment Station. RB07 was released as a cultivar in 2007 on the basis of its high and consistent grain yield, earliness, resistance to wheat leaf rust (caused by *Puccinia triticina* Eriks.), moderate resistance to Fusarium head blight (caused primarily by *Fusarium graminearum* Schwabe), and good grain end-use quality (Anderson et al. 2009). The F_6-derived line MN06113-8 has the pedigree MN97695-Lr52/HJ98-Fhb1 and is a breeding line from the University of Minnesota Wheat Breeding Program that was advanced to second year yield trials before being discontinued for consideration as a new cultivar candidate. Seed increases in the F_6 lines were done in a greenhouse to obtain enough seeds for field phenotyping. Seedling tests for reaction of RB07 and MN06113-8 to races TTKSK (isolate 04KEN156/04), TTKST (isolate 06KEN19v3), and TTTSK (isolate 07KEN24-4) were carried out by following Rouse and Jin (2011) at the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory. Infection types (ITs) were recorded on a 0–4 scale according to Stakman et al. (1962).

Field stem rust evaluation

The $F_{6:7}$ and $F_{6:8}$ populations, along with the parents, were evaluated for their field response to African stem rust races at two locations in East Africa over three seasons: at Njoro, Kenya, during the 'main season' from June to October of 2012 and the 'off-season' from January to April 2013 (referred to as Ken12 and Ken13 hereafter, respectively) and at Debre Zeit, Ethiopia, during the 'off-season' from January to June of 2013 (hereafter referred to as Eth13). The population was also evaluated in St. Paul, MN, USA, during May to August 2013 (hereafter referred as StP13) for field response to North American stem rust races.

In the Njoro nursery (Ken12 and Ken13), lines were planted in an augmented design with the susceptible check line 'Red Bobs' (Cltr. 6255) planted after every fifty entries. Each line was sown in double 70-cm-long rows, 20 cm apart. On each side of the plot, and in the middle of the plots, a twin row of susceptible spreader

wheat cultivar 'Cacuke' was sown. The field was also surrounded by a border of several spreader rows comprised of susceptible wheat varieties that were artificially inoculated using a bulk inoculum of *Pgt* urediniospores collected at the Njoro field site. Wheat stem rust differential lines with known stem rust resistance genes indicated that the predominant, if not only, *Pgt* race present in the nursery since 2008 was race TTKST (avirulence/virulence formula on the wheat stem rust differential panel: *Sr36*, *SrTmp*/*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr17*, *Sr21*, *Sr24*, *Sr30*, *Sr31*, *Sr38*, *SrMcN*; Njau et al. 2010).

In the Debre Zeit nursery (Eth13), lines were planted in 1-m-long twin rows that were flanked by spreader rows comprised of a mixture of susceptible wheat lines 'PBW343,' 'Morocco,' and 'Local Red.' The RILs were planted in an augmented design with the susceptible check line 'Red Bobs' planted after every fifty entries. To initiate the disease, spreader rows were artificially inoculated with bulk inoculum of fresh *Pgt* urediniospores collected locally from wheat cultivar 'PBW343' and urediniospores also collected from local fields. PBW343 contains the gene *Sr31*, and several races in the Ug99 race group are virulent to *Sr31*, whereas all other known *Pgt* isolates are avirulent.

In the St. Paul nursery, lines were planted in hill plots with 20 cm distance between the hills. The population was planted in an augmented design with four check varieties 'Oklee' (Anderson et al. 2005), 'Thatcher' (Hayes et al. 1936), 'Tom' (Anderson et al. 2012), and 'Verde' (Busch et al. 1996) planted after every 30 entries. A mixture of susceptible lines 'Morocco' and 'LMPG-6' were planted perpendicular to surround the lines on all sides. To initiate disease, spreader rows were syringe-injected with a mixture of North American *Pgt* races MCCFC (isolate 59KS19), QFCSC (isolate 03ND76C), QTHJC (isolate 75ND717C), RCRSC (isolate 77ND82A), RKQQC (isolate 99KS76A), and TPMKC (isolate 74MN1409) at the jointing stage. The spreader rows were also sprayed with a bulked mixture of the six *Pgt* races suspended in a light mineral oil suspension using an Ulva + sprayer (Micron Sprayers Ltd, Bromyard, UK) after heading.

Phenotyping and data analysis

Field reaction of the RILs to stem rust were recorded as disease severity on the 0–100 modified Cobb scale (Peterson et al. 1948), and infection response, based on the size of pustules and amount of chlorosis and necrosis visible on the stem (Roelfs et al. 1992). Phenotyping of the population segregating for resistance was carried out after the susceptible check varieties in each trial had attained maximum disease severity. Following Stubbs et al. (1986), the severity response value was multiplied with the infection response to obtain coefficient of infection values. Growth stages of the lines were used as covariates in a mixed model in *lme4* (R 3.0.3, R Development Core Team, 2013) to obtain phenotypic values corrected for differences in growth stage among the lines. Growth stages of the lines were determined mainly by assessing grain development stages, such as watery, milky, soft dough, and hard dough, and also for stages of booting and flowering, as explained by Zadoks et al. (1974). Phenotypic values corrected for growth stage differences were used to perform analysis of variance (ANOVA) in SAS 9.1 (SAS Institute Inc, Cary, NC, USA). Using the function PROC GLM, genotypes were modeled as random effects and locations (Kenya, Ethiopia, St. Paul) and trials (Ken12, Ken13, Eth13, StP13) as fixed effects. Thus obtained best linear unbiased predictors (BLUPs) were used to map QTL. Replicated checks were used to calculate the pooled error mean square value. Pearson correlation coefficients among the trials were calculated using the function PROC CORR in SAS 9.1.

Molecular marker assay

Genomic DNA was extracted from ground seeds of the parents and $F_{6:7}$ RILs using a modified cetyl trimethylammonium bromide (CTAB) protocol (Kidwell and Osborn 1992). The extracted DNA was quantified using an ND 1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA). The population was genotyped using SNP markers obtained from two approaches: (1) the 9000 Infinium iSelect SNP assay (9K; Cavanagh et al. 2013) and (2) genotyping by sequencing (GBS; Elshire et al. 2011).

For genotyping using the Infinium iSelect assay, DNA suspended in ddH_2O at approximately 80 ng/µl was submitted to the USDA-ARS Small Grain Genotyping Center, Fargo, ND, USA. The data generated were manually called using Illumina's GenomeStudio 2011.1 (Illumina Inc, Hayward, CA, USA). Briefly, monomorphic markers (markers with the same calls

for the entire population), markers with more than 10 % missing data, and markers that deviated from a 1:1 segregation ratio were discarded. Markers with 5 % or less heterozygous calls were retained to avoid false purging of heterozygous loci. This resulted in 1050 high-quality markers that were retained for linkage mapping.

To increase mapping resolution, and partly to investigate the feasibility of genome mapping using markers obtained from next-generation sequencing, the population was also genotyped using the GBS method (Elshire et al. 2011). In the GBS approach, a double-digested library was created using the restriction enzymes *Pst*I and *Msp*I on 200 ng of DNA per sample, following Poland et al. (2012) with modifications. Each library was 76-plexed, with the parents repeated six times each, and the libraries were sequenced in two lanes of Illumina HiSeq 2000, generating 100-bp paired-end sequences. The sequences were processed using the UNEAK pipeline (Lu et al. 2013) using the parameters -c 10 −e 0.025 to obtain GBS SNPs. Reads containing SNPs were used as query sequences and BLASTN-searched against the wheat chromosome survey sequences (CSS) to assign SNPs to unique chromosomes. The wheat CSS were obtained by assembling reads obtained from sequencing flow-sorted wheat chromosomes from the 'Chinese Spring' variety (International Wheat Genome Sequencing Consortium, http://wheaturgi.versailles.inra.fr/Seq-Repository/). To ensure that correct SNPs were obtained, only the full-length alignment of a query sequence with the survey sequences allowing either one base mismatch or one gap was permitted. To circumvent retaining of redundant SNPs on paralog sequences and duplicated regions among the A, B, and D subgenomes, SNPs thus obtained were filtered to remove those that mapped more than once to multiple chromosomes. SNPs that were monomorphic, had no allele calls for >10 individuals (>7 % missing data), and were heterozygous in >10 individuals (7 % heterozygosity) were also discarded. This process resulted in 932 high-quality SNP markers that were retained for linkage mapping. Sequences and allele types of these GBS SNPs are available in Supplementary File 1.

Linkage map construction and QTL mapping

SNPs obtained from both genotyping approaches (9K, GBS) were combined to assign markers to linkage groups. Linkage groups were constructed using Mapdisto V1.7.7.0.1 (Lorieux 2012) using a minimum logarithm of odds (LOD) value of 3.0. Genetic distances between the markers were calculated based on the Kosambi mapping function (Kosambi 1943). The program Windows QTL Cartographer 2.5_011, which implements composite interval mapping (CIM) to identify QTL, was used to analyze marker–trait associations (Wang et al. 2012). The LOD threshold for declaring a significant QTL was calculated by 1000 permutations at $\alpha = 0.05$ and set at 2.5. A walk speed of 1 cM was used for QTL detection. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by the QTL. If multiple QTL were detected in an environment, digenic additive x additive epistatic interactions were tested among the detected QTL using the multiple interval mapping (MIM) algorithm available in the same program. Using multiple marker intervals simultaneously, the MIM procedure fits multiple putative QTL directly in the QTL mapping model and estimates several genetic architecture parameters including the effects of and interactions among significant QTL. Epistatic interaction among all SNP markers, irrespective of their association with the detected QTL, was also carried out using the MIM algorithm. A QTL × QTL interaction was declared significant if the LOD threshold was ≥1.0.

Results

Disease evaluation

Both parents conferred medium to high levels of resistance in the field in Kenya (Table 1), but were seedling susceptible to TTKSK, TTKST, TTTSK races (IT 3+ for both lines to all three races). The disease pressure observed in each environment was adequate for good discrimination among stem rust phenotypes of the RILs and the mapping of loci associated with quantitative resistance. The disease severity distributions skewed toward the lower percent severity responses overall (Fig. 1). The highest disease pressure was observed in Ethiopia in 2013 with lines showing up to 70 % severity. Disease scores recorded for the RIL population along with the parents in all four environments are presented in Table 1. There was no significant difference between MN06113-8 and RB07 for their average stem rust

Table 1 Mean values and range of stem rust severity (%) in the RIL mapping population derived from RB07/MN06113-8 in four field environments at Njoro, Kenya, Debre Zeit, Ethiopia, and St. Paul, MN, USA

Environments	Parent disease severity (%) (mean ± SD)		Parent growth stage[a]		RIL population	
	MN06113-8	RB07	MN06113-8	RB07	Mean ± SD	Range
Kenya 2012	1.0 ± 0.0	14.4 ± 3.8	85	85	23.8 ± 13.8	1.0–50.0
Kenya 2013	20.8 ± 8.8	23.3 ± 1.4	75	65	30.5 ± 8.9	12.5–45.0
Ethiopia 2013	42.5 ± 2.9	51.3 ± 4.8	–	–	47.2 ± 12.2	15.0–70.0
St. Paul 2013	16.7 ± 2.9	16.7 ± 2.9	85	85	20.6 ± 7.4	5.0–40.0
Mean	20.3 ± 3.6	26.4 ± 3.2			30.5 ± 10.6	8.4–51.3

[a] Growth stages were assigned according to Zadoks et al. (1974)

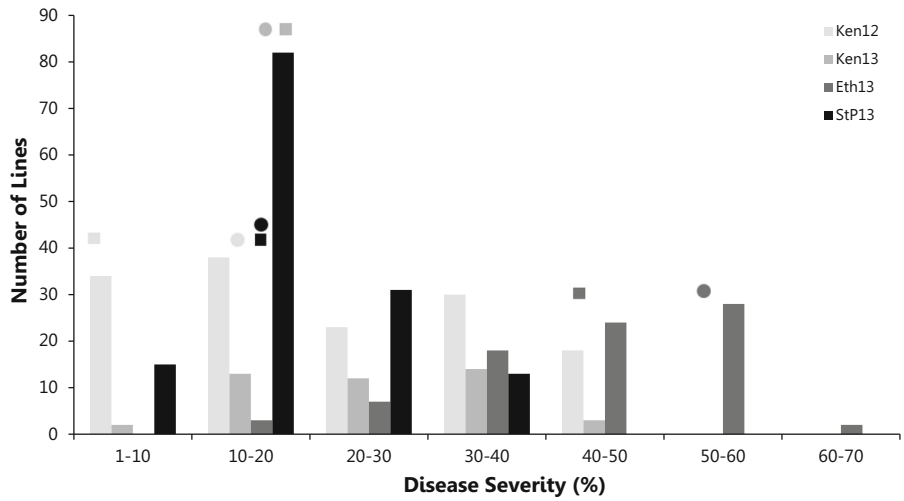

Fig. 1 Frequency distribution of stem rust severity (%) for the RB07/MN06113-8 population comprised of 141 recombinant inbred lines evaluated in four environments: two at Njoro, Kenya, one at Debre Zeit, Ethiopia, and one at St. Paul, MN, USA. *Square and circle symbols* (with the *same color* scheme as the *bars*) represent the average disease severity of the parents MN06113-8 and RB07, respectively, in each environment. *Ken12* Kenya 2012, *Ken13* Kenya 2013, *Eth13* Ethiopia 2013, *StP13* St. Paul 2013

responses across all environments (*t* test *P* value of 0.25 at $\alpha = 0.05$). This is not completely unexpected, as both parent lines exhibit similar levels of APR in the field (Table 1; Fig. 1). When differences were observed, such as in the three African environments, MN06113-8 displayed a lower severity compared to RB07. Disease severity distributions for the RILs across all environments were continuous, suggestive of quantitative and polygenic resistance. Disease reactions of RILs between Kenya and Ethiopia nurseries were strongly correlated, whereas correlations with the St. Paul reactions were lower, yet still significant (Table 2). The lower correlation

Table 2 Pearson correlation coefficients of stem rust severity observed in the RB07/MN06113-8 population in four field environments at Njoro, Kenya, Debre Zeit, Ethiopia, and St. Paul, MN, USA

	Ken12	Ken13	Eth13
Ken13	0.40		
Eth13	0.55	0.66	
StP13	0.32	0.29	0.48

Ken12 = Njoro, Kenya 2012; Ken13 = Njoro, Kenya 2013; Eth13 = Debre Zeit, Ethiopia 2013; StP13 = St. Paul, MN 2013

All correlations are significant at $\alpha < 0.001$

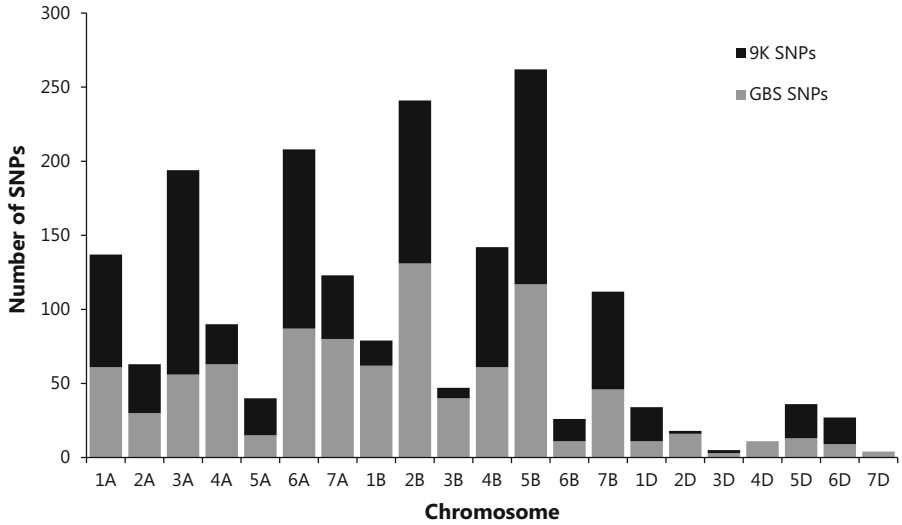

Fig. 2 Distribution of markers (both 9K SNPs and GBS SNPs) across the 21 wheat chromosomes. Markers from multiple linkage groups were pooled if a chromosome had more than one linkage group

coefficients between St. Paul and the Kenyan environments suggest the presence of genotype by environment (G×E) interaction, corroborated by the significant F test value of 3.1 (significant at $P < 0.001$) in the combined ANOVA. Some of the factors that could have contributed toward G×E could be the differences between *Pgt* races, inoculum load, and differences in environmental conditions.

Construction of linkage maps of the RIL population

SNPs obtained from both genotyping methods—the 9K SNP chip and de novo GBS SNPs—were combined to develop linkage maps to represent wheat chromosomes. Of the 1982 markers used for linkage mapping, 1899 (972 9K, 927 GBS) were assigned to 29 linkage groups. All 21 wheat chromosomes were represented by the linkage groups, with 8 chromosomes represented by two linkage groups. These markers covered 1950 cM of the genome, with an average interval of 1.03 cM. Most SNPs were assigned to the A and B genomes, with 855 and 909 SNPs, respectively, while 135 SNPs were assigned to the D genome (Fig. 2).

Quantitative mapping of resistance to stem rust

The CIM method of QTL mapping detected six significant QTL involved in APR in the African environments and four QTL involved in APR in St. Paul (Table 3) with one QTL significant in all four environments. The detected QTL ranged in their LOD scores from 2.6 to 16, with four QTL contributed by MN06113-8 and five contributed by RB07. The QTL *QSr.umn-2B.2* was detected in all environments (Fig. 3) and explained the observed phenotypic variation (R^2) as follows: 31.4 % in Ken12, 27.1 % in Ken13, 46.9 % in Eth13, and 8.7 % in StP13. This QTL, derived from MN06113-8, had the largest effect in all environments except in StP13 where the QTL *QSr.umn-4B.2* was the largest in terms of both R^2 and allelic effect (Table 3). Other QTL explained 5–13 % of the resistance variation, but were significant only in one of the four environments. Multiple QTL on a single chromosome were detected in two environments: Ken12 with two QTL on chromosome 2B and StP13 with two QTL on chromosome 4B.

Test for additive × additive epistasis among the detected QTL revealed no significant interactions. Test for epistasis among all loci (significant or not) also detected no significant interactions. All two QTL models were generated to estimate the average reduction in severity (Table 4). The results indicate that combinations of at least two QTL in a gene-pyramiding scheme reduced disease severity values from 9.2 to 52.2 % in the environments where the QTL were detected.

Discussion

In this study, we report several genomic regions associated with APR to African *Pgt* races in the Ug99 race group and North American *Pgt* races in lines derived from the University of Minnesota wheat

Table 3 Quantitative trait loci (QTL) for APR to stem rust detected in the RB07/MN06113-8 population of 141 recombinant inbred lines by composite interval mapping in four environments

Environment[a]	QTL[b]	Chr	Flanking markers[c]		Pos (cM)	LOD[d]	R^{2e}	Add[f]
			Left	Right				
Ken12	QSr.umn-2B.1	2BL	TP3836	TP10865	15.3	4.7	7.6	−3.0
	QSr.umn-2B.2	2BS	wsnp_Ex_rep_c71023_69867676	TP38912	96.9	16.0	31.4	6.1
	QSr.umn-6D	6DS	wsnp_BE445201D_Ta_1_1	TP16823	0.01	3.4	5.4	−2.5
	QSr.umn-7A	7AS	TP2402	TP2424	0.8	2.9	4.5	−2.3
Ken13	QSr.umn-1A	1AS	wsnp_Ku_c5756_10191339	TP33660	98.3	2.9	12.5	3.6
	QSr.umn-2B.2	2BS	wsnp_Ex_rep_c71023_69867676	TP38912	99.3	4.6	27.1	5.2
	QSr.umn-2D	2DS	TP8148	TP26641	33.6	3.1	13.1	−3.6
Eth13	QSr.umn-2B.2	2BS	wsnp_Ex_rep_c71023_69867676	TP38912	97.9	13.5	46.9	10.0
StP13	QSr.umn-2B.2	2BS	wsnp_Ex_rep_c71023_69867676	TP38912	104.7	4.1	8.7	2.5
	QSr.umn-4A	4AL	TP10795	wsnp_Ex_c2352_4405961	20.9	2.7	5.6	2.0
	QSr.umn-4B.1	4BS	wsnp_Ex_c6739_11646407	wsnp_Ku_c8075_13785546	21.7	2.8	5.9	−2.2
	QSr.umn-4B.2	4BL	wsnp_Ku_c8075_13785546	TP4428	43.1	5.6	12.3	3.4

[a] Ken12 = Njoro, Kenya 2012; Ken13 = Njoro, Kenya 2013; Eth13 = Debre Zeit, Ethiopia 2013; StP13 = St. Paul, MN 2013

[b] QTL were named according to McIntosh et al. (2003)

[c] Markers beginning with 'TP' are GBS SNPs discovered from GBS approach

[d] LOD values are the peak logarithm of odds score for the given QTL

[e] Value indicates the phenotypic variation explained by the QTL

[f] Value indicates the estimated additive effect of the QTL; negative value means that the allele was contributed by RB07

breeding program. Analysis of the segregating population in four environments led to identification of nine QTL on chromosomes 1A, 2B, 2D, 4A, 4B, 6D, and 7A, explaining from 4.5 to 46.9 % of resistance variation in the field.

The QTL *QSr.umn-2B.2* was significant in all four environments and was contributed by the resistant parent MN06113-8 (Table 3). A small-effect QTL, *QSr.umn-2B.1*, was also detected on chromosome 2B in the Ken12 environment. Based on mapping of significant SNP sequences to the wheat CSS and also the published 9K map (Cavanagh et al. 2013), the chromosomal locations of *QSr.umn-2B.1* and *QSr.umn-2B.2* were determined to be on the long arm and short arm of 2B, respectively. QTL located on 2B that provide resistance to African stem rust races have been reported previously as summarized by the Ug99 resistance loci consensus map including QTL mapped to 2B from unpublished the International Center for the Improvement of Maize and Wheat (CIMMYT) populations (Yu et al. 2014). Singh et al. (2013a) reported *QSr.cim-2BS* on 2BS between the Diversity Arrays Technology (DArT) markers *wPt-9230* and *wPt-744022* that explained 3.2–6.2 % of the resistance expressed in the CIMMYT population PBW343/Muu. Bhavani et al. (2011) also detected a QTL on chromosome 2B between the DArT markers *wPt-7829* and *wPt-2266* that provided moderate to low levels of resistance to Ug99 races in the CIMMYT population PBW343/Juchi. Additionally, in an association analysis of CIMMYT spring wheat germplasm, Yu et al. (2011) reported that the markers *wPt-7750*, *wPt-8460*, and *wPt-7200* on chromosome 2B were significantly associated with resistance to races of the Ug99 lineage. Crossa et al. (2007) detected a non-Ug99 stem rust QTL associated with the 2BS markers *wPt-0100* and *wPt-4916*, in the same region as *QSr.umn-2B.2*, in CIMMYT's elite spring wheat germplasm. We used the integrated genetic map consisting of different marker types generated by Maccaferri et al. (2015) to compare the QTL positions in our study with significant markers reported in previous studies and found that the marker *wPt-7750* to be the closest to the *QSr.umn-2B.2* peak, at a distance of 34 cM. Thus, neither of these reported markers nor the mapped locations of the seedling genes on 2B (*Sr9h*, *Sr28*, *Sr36*, *Sr39*, *Sr40*, and *Sr47*) that provide resistance to the Ug99 race group (Hiebert

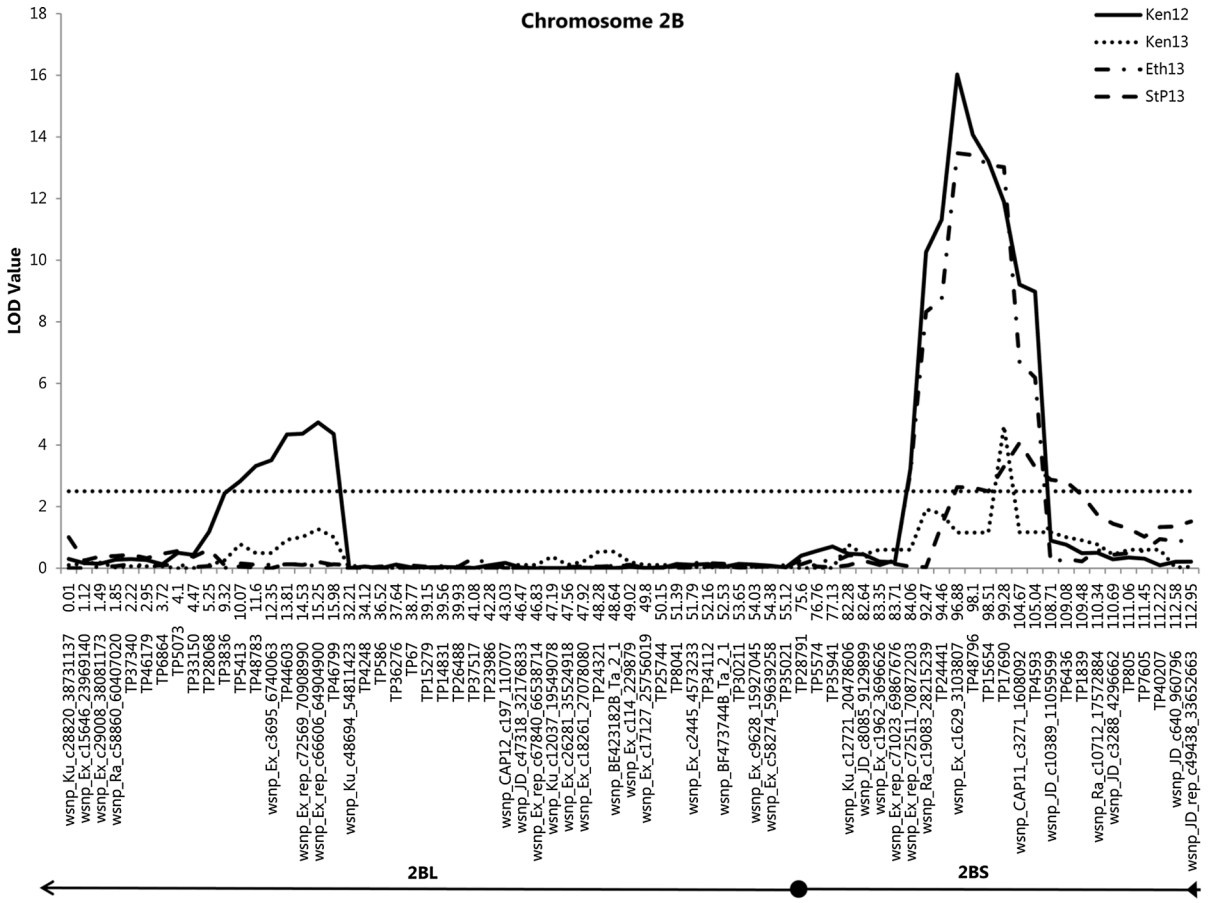

Fig. 3 Quantitative trait loci (QTL) interval map for *QSr.umn-2B.2* detected on wheat chromosome 2B. The QTL is associated with stem rust resistance in the RB07/MN06113-8 population of recombinant inbred lines and provided resistance in all environments. The *Y-axis* indicates the logarithm of odds (LOD) values with the *dotted line* representing the threshold LOD score of 2.5. The *X-axis* is labeled with SNP markers and the genetic distances in centimorgan (cM) between the markers. Markers beginning with 'TP' are SNPs discovered from GBS approach. The *arrowed line* shows the orientation of the linkage map, with the *circle* representing the centromere. *Ken12* Kenya 2012, *Ken13* Kenya 2013, *Eth13* Ethiopia 2013, *StP13* St. Paul 2013

et al. 2010; Klindworth et al. 2012; Wu et al. 2009; Tsilo et al. 2008; Niu et al. 2011; Rouse et al. 2012; 2014a) overlap or closely flank the QTL region on 2BS reported in this study.

No known major genes effective to Ug99 races are postulated in the population as the parents are susceptible at the seedling stage to the races TTKSK, TTKST, and TTTSK with IT 3+ to these races. Therefore, the QTL *QSr.umn-2B.2* on 2BS could be an important discovery with a relatively large effect on disease reduction. More importantly, this QTL was detected in all environments we used to screen the population, suggesting that it is effective to all of the races used in these environments. Although, it is possible that the QTL may not be effective to one or more races that is present at a low frequency in the disease nursery, such as in the St. Paul nursery. Screening of the population in single-race nurseries would elucidate the efficacy of this QTL to the races. Regardless, the use of *QSr.umn-2B.2* in breeding for APR to stem rust globally could be a major advantage in the fight against wheat stem rust. In addition, some wheat APR genes are known to be effective against more than one pathogen, either due to pleiotropy or due to colocalization of genes resistant to multiple pathogens (Risk et al. 2013; Suenaga et al. 2003; William et al. 2003). The short arm of chromosome 2B reportedly contains APR QTL conferring resistance to

Table 4 Reduced stem rust severity in the RB07/MN06113-8 RIL population by combinations of QTL in pairs in each environment

Environment	QTL combinations	Stem rust severity (%)		Difference in severity	Disease reduction (%)
		No QTL[a]	2 QTL combination[a]		
Ken12	2B.1 + 2B.2	29.7	16.6	13.1	44.2
	2B.1 + 6D	29.1	17.6	11.5	39.6
	2B.1 + 7A	28.5	15.7	12.9	45.1
	2B.2 + 6D	28.3	13.5	14.8	52.2
	2B.2 + 7A	29.4	14.7	14.7	50.0
	6D + 7A	31.8	17.9	13.9	43.8
Ken13	1A + 2B.2	34.3	24.7	9.6	28.0
	1A + 2D	34.1	21.5	12.6	37.0
	2B.2 + 2D	35.5	24.0	11.5	32.4
StP13	2B.2 + 4A	23.4	17.4	6.0	25.8
	2B.2 + 4B.1	21.2	17.9	3.3	15.6
	2B.2 + 4B.2	23.3	18.1	5.2	22.2
	4A + 4B.1	24.4	17.9	6.5	26.6
	4A + 4B.2	20.6	18.7	1.9	9.2
	4B.1 + 4B.2	25.2	19.1	6.1	24.1
Africa[b]	2B.2	33.3	25.6	7.7	30.0
USA[b]	2B.2	21.6	19.4	2.2	10.4
All environments[c]	2B.2	29.4	23.9	5.5	18.7

The environment Ethiopia 2013 is not shown because only 1 QTL was detected in this environment

[a] Numbers represent the average disease severity (%) of genotypes upon absence of QTL and combining two QTL

[b] The effect of the presence/absence of the uniform QTL *QSr.umn-2B.2* on stem rust severity in Africa and US environments

[c] The effect of the presence/absence of the uniform QTL *QSr.umn-2B.2* on stem rust severity in all environments. Stem rust severity scores for the genotypes were averaged across the four environments

stripe rust (Carter et al. 2009; Prins et al. 2011) and leaf rust (Tsilo et al. 2014). Therefore, it may be worth investigating whether the QTL *QSr.umn-2B.2* also provides resistance to other pathogens such as leaf rust and stripe rust.

The minor effect QTL *QSr.umn-1A* and *QSr.umn-2D* were both detected in the Ken13 environment. Other loci on chromosome 1A that are significantly associated with resistance to African stem rust races have been reported by Rouse et al. (2014b) in 'Thatcher' wheat (QTL *QSr.cdl-1AL*), Bhavani et al. (2011) in the CIMMYT biparental population PBW343/Kingbird (QTL between the markers *wPt-0128* and *wPt-734078*), Yu et al. (2012) in CIMMYT's winter wheat breeding germplasm, Pozniak et al. (2008) in a durum wheat (*Triticum durum* Desf.) association mapping panel, and Singh et al. (2013b) in the durum wheat population Sachem/Strongfield. The only QTL located on chromosome 2D providing APR to the African stem rust races was reported by Bhavani et al. (2011) in the CIMMYT population PBW343/Kiritati, which is located 14 cM from the position of *QSr.umn-2D*.

Another minor effect QTL observed in our study was *QSr.umn-4A*, located on the short arm of chromosome 4A. Previous reports of QTL located on 4A include the mapping studies by Bhavani et al. (2011) and Yu et al. (2011). The QTL *QSr.umn-4B.1* and *QSr.umn-4B.2* were both detected in the StP13 environment, yet the former was contributed by RB07 and the latter by MN06113-8. Previous reports of QTL on 4B include a minor effect QTL detected by Bhavani et al. (2011) in the PBW343/Kingbird population; QTL contributed by the Canadian cultivar 'Carberry' (Singh et al. 2013a); and an APR QTL contributed by the Indian cultivar 'WL711' in the RIL population HD2009/WL711 (Kaur et al. 2009).

The QTL *QSr.umn-6D* was detected only in the Ken12 environment, yet could be novel as no QTL effective to the Ug99 races have been reported in this

region. This QTL originates from RB07 and is located on the short arm of chromosome 6D. The all-stage resistance gene *Sr42* is also located on 6DS and provides resistance to the African races TTKSK, TTKST, and TTTSK (Ghazvini et al. 2012). Since both parents showed susceptible infection types to these three races during seedling screening and were negative for the presence of *Sr42* during marker screening, *Sr42* may not be present in either parent. Another all-stage resistance gene present on 6DS, *Sr5*, is not effective against races in the Ug99 lineage (Singh et al. 2011) and does not provide APR to stem rust when deployed singly, but it may be involved in APR when used with other genes (Nazareno and Roelfs 1981; Knott 2001). We do not have sufficient information to investigate the relationship between *QSr.umn-6D* and *Sr5*.

The QTL *QSr.umn-7A*, also contributed by RB07, mapped on the distal end of chromosome 7AS (0.8 cM) and explained 4.5 % of the phenotypic variance. Singh et al. (unpublished; see Yu et al. 2014) reported a QTL located between 2.9 and 5.6 cM on chromosome 7AS in the PBW343/Kenya Nyangumi population, which could be the same as the QTL detected in our study. Further studies including fine mapping of the region are essential to establish the novelty of this resistance locus. Also, given that we implemented SNP markers and other studies have used either DArT or SSR markers, a satisfactory direct comparison with published QTL cannot be made with existing information. Characterizing the QTL detected in our study in relation to QTL identified in other studies can be helpful in terms of sustainable gene deployment strategies. However, the use of different marker systems in different studies makes it difficult to do so. Regardless, description of these QTL within breeding germplasm can aid significantly in the fight against the disease.

One interesting discovery made in this study is the lack of detection of QTL on chromosomes with genes exhibiting pleiotropic APR to stem rust and other diseases, as reported in published studies. No QTL were detected on chromosomes 1B (location of *Sr58*), 3B (location of *Sr2*), 4D (location of *Sr55*), 5B (location of *Sr56*), and 7D (location of *Sr57*). The lack of QTL detection on these chromosomes suggests that a previously undetected APR gene could be present in the RB07/MN06113-8 population. However, the marker density on chromosomes 4D and 7D (11 and 4 markers, respectively) in our study limited our ability to potentially detect *Sr55* or *Sr57*. Screening for the presence of *Sr57* (colocalized with *Lr34/Yr18/Pm38*) using the STS marker *csLV34* developed by Lagudah et al. (2006) showed that neither parent carried *Sr57*. Screening of other APR genes *Sr55*, *Sr56*, and *Sr58* was not possible due to unavailability of diagnostic markers. Our discovery of *QSr.umn-2B.2* in all four environments, as well as other QTL in previously unreported chromosomal locations, provides a strong case for the existence of new QTL in the RB07/MN06113-8 population that confer APR against African and North American stem rust races. Identification of new APR genes is significant because it would provide breeders with new and possibly durable tools to develop varieties resistant to highly virulent *Pgt* races such as the Ug99 race group. The discovery of new APR genes and their deployment can also alleviate the selection pressure put on the pathogen by newly deployed race-specific resistance genes (Evanega et al. 2014; Singh et al. 2008) and therefore prolong their durability. Further field screening and tests are required to confirm the novelty of these discovered regions. Also, identifying markers linked to these QTL would aid in marker-assisted selection to enhance resistance.

The emergence of widely virulent African stem rust races is often credited to the pathogen's ability to defeat singly deployed resistance genes in areas conducive to the pathogen's growth, development, and evolution. To slow down the pathogen from developing virulence by single-step mutations for deployed resistance genes, the scheme of resistance breeding by pyramiding multiple genes has been proposed (Knott 1989). As pyramiding of multiple genes in a single line can be resource intensive given the large number of progeny needed for screening (Bonnett et al. 2005), combining fewer genes may be desirable to obtain immediate resistance to the disease. The combination of only two QTL detected in our study showed clear reduction in disease severity (Table 4) and can be implemented to lower the disease pressure. Combining multiple QTL as the long-term breeding goal could be of greater interest, especially in disease hotspots, as pyramiding APR genes with major genes may also increase the durability of major genes (Burdon et al. 2014; Mundt 2014). Further work to fine map the regions and to identify diagnostic markers is required to accomplish successful pyramiding of multiple QTL.

Conclusions

It is generally considered that the widely virulent African stem rust races reaching the breadbaskets of Asia and the Americas is a real possibility (Hodson et al. 2011). Understanding the threat of their possible arrival, several mapping studies in different types of mapping populations have been conducted to discover resistance loci effective against the Ug99 race group. Here, we report a large-effect APR QTL, *QSr.umn-2B.2*, and other sources of resistance that are effective against African and North American *Pgt* races. The RIL mapping population was developed using advanced breeding lines from the University of Minnesota wheat breeding program. An advantage of mapping resistant QTL in a population obtained from crossing two elite parent lines is that the resistant line can be used as a donor parent in a recurrent breeding scheme without linkage drag. The discovery of these QTL in our elite parent lines should offer value to their utilization for APR to the Ug99 race group in other breeding programs globally.

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Author contribution P.B. supervised planting of the RIL population in St. Paul, MN; recorded the phenotype of the RIL population in St. Paul, MN; carried out genotyping of the plant materials and data analysis; and drafted the manuscript. M.N.R. recorded the phenotype in East African nurseries and assisted in preparing the manuscript. S.B. supervised planting of and disease inoculation on the population in East African rust nurseries. J.A.A. conceived the study, developed the population, supervised the project, and assisted in preparing the manuscript. All authors contributed to and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Human participants and/or animals statement Not applicable.

Informed consent Not applicable.

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